

101522074

Fig.1

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<u>Pre</u>	<u>Pro</u>	-----pre-albumin-----  -----pro region-----
HSA	HSA	Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala Tyr Ser Arg Gly Val Phe Arg Arg
HSA	MFA-1	Met Lys Trp Val Thr Phe Ile Ser Leu Leu Phe Leu Phe Ser Ser Ala Tyr Ser Arg Ser Leu Asp Lys Arg
		-24 -23 -22 -21 -20 -19 -18 -17 -16 -15 -14 -13 -12 -11 -10 -9 -8 -7 -6 -5 -4 -3 -2 -1
	Preferred mutations of the invention:	Phe Ile Val Ile

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**Fig.2**Standard genetic code

	T	C	A	G
T	TTT Phe (F) TTC Phe (F) TTA Leu (L) TTG Leu (L)	TCT Ser (S) TCC Ser (S) TCA Ser (S) TCG Ser (S)	TAT Tyr (Y) TAC Tyr (Y) TAA Ter TAG Ter	TGT Cys (C) TGC Cys (C) TGA Ter TGG Trp (W)
C	CTT Leu (L) CTC Leu (L) CTA Leu (L) CTG Leu (L)	CCT Pro (P) CCC Pro (P) CCA Pro (P) CCG Pro (P)	CAT His (H) CAC His (H) CAA Gln (Q) CAG Gln (Q)	CGT Arg (R) CGC Arg (R) CGA Arg (R) CGG Arg (R)
A	ATT Ile (I) ATC Ile (I) ATA Ile (I) ATG Met (M)	ACT Thr (T) ACC Thr (T) ACA Thr (T) ACG Thr (T)	AAT Asn (N) AAC Asn (N) AAA Lys (K) AAG Lys (K)	AGT Ser (S) AGC Ser (S) AGA Arg (R) AGG Arg (R)
G	GTT Val (V) GTC Val (V) GTA Val (V) GTG Val (V)	GCT Ala (A) GCC Ala (A) GCA Ala (A) GCG Ala (A)	GAT Asp (D) GAC Asp (D) GAA Glu (E) GAG Glu (E)	GGT Gly (G) GGC Gly (G) GGA Gly (G) GGG Gly (G)

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Single letter code:

A = adenosine

C = cytidine

G = guanosine

10 T = thymidine

B = C or G or T

D = A or G or T

H = A or C or T

K = G or T

15 M = A or C

N = A or C or G or T

R = A or G

S = C or G

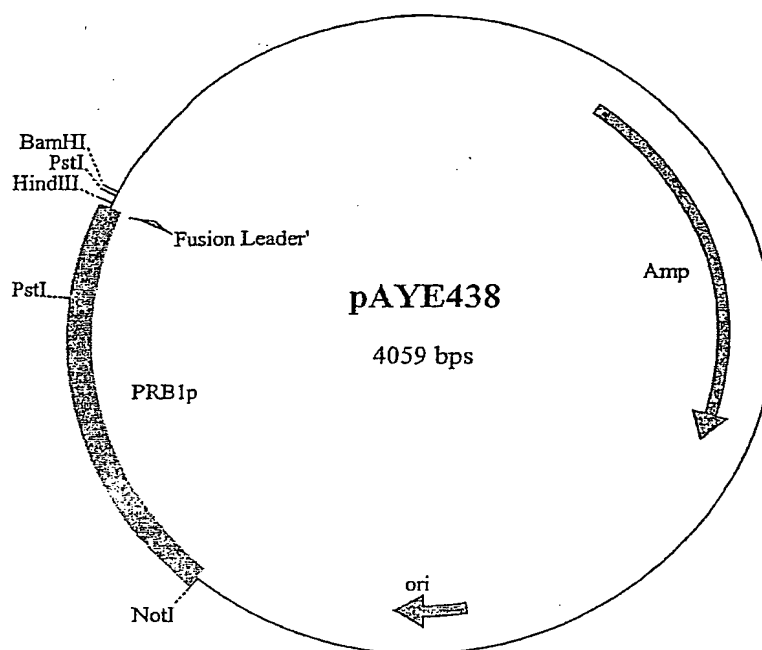
V = A or C or G

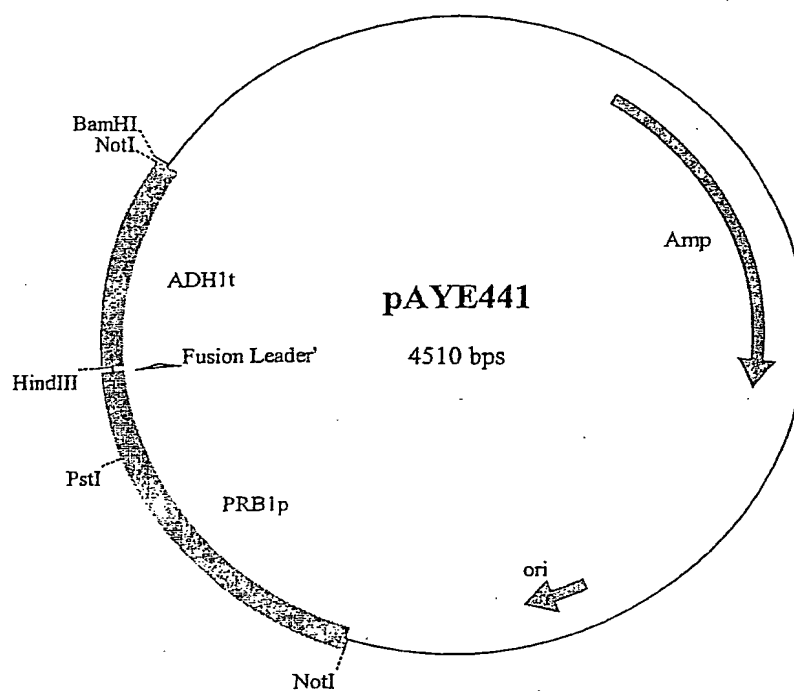
20 W = A or T

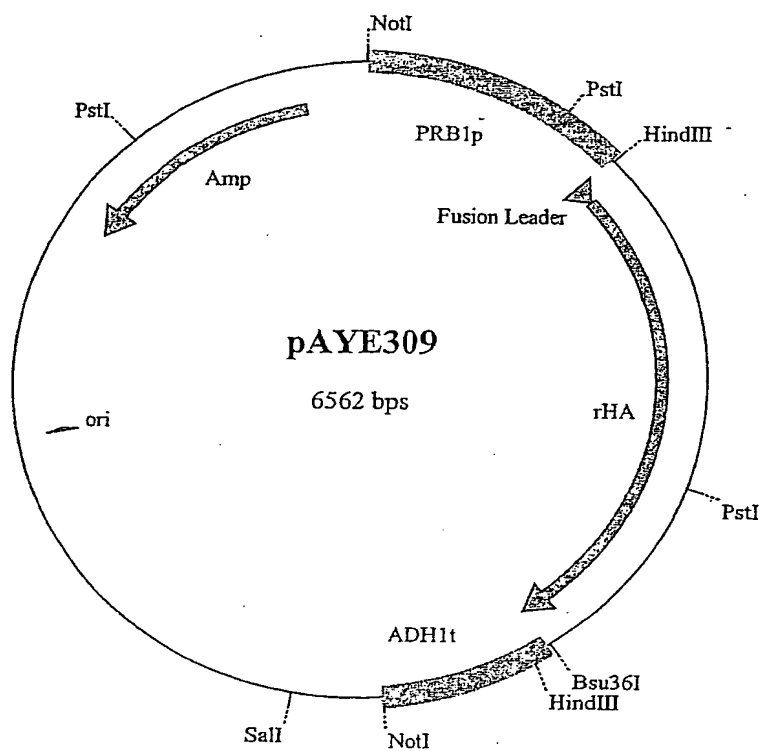
Y = C or T

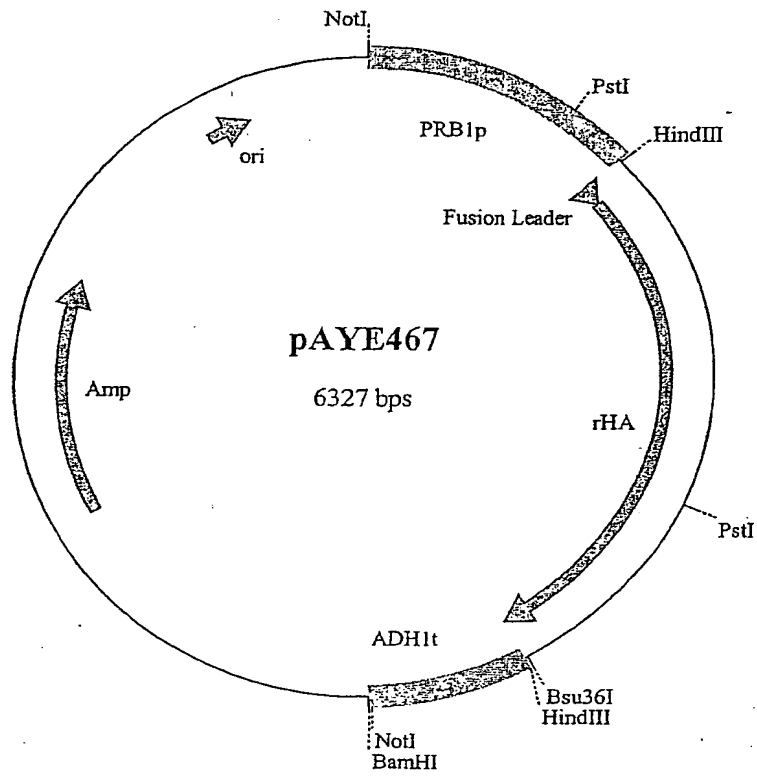
*Fig.3*Modified list of preferred yeast codons

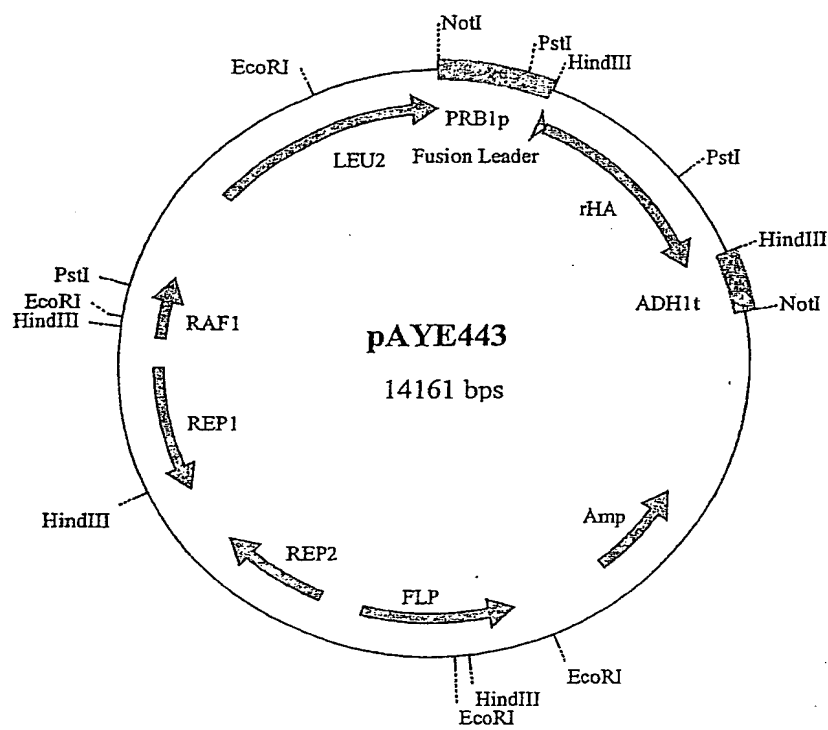
	T	C	A	G
T	TTC Phe (F) TTG Leu (L)	TCT Ser (S) TCC Ser (S)	TAC Tyr (Y) TAA Ter	TGT Cys (C) TGG Trp (W)
C		CCA Pro (P)	CAT His (H) CAA Gln (Q)	
A	ATT Ile (I) ATC Ile (I) ATG Met (M)	ACT Thr (T) ACC Thr (T)	AAC Asn (N) AAG Lys (K)	AGA Arg (R)
G	GTT Val (V) GTC Val (V)	GCT Ala (A)	GAT Asp (D) GAC Asp (D) GAA Glu (E)	GGT Gly (G)

*Fig.4*

*Fig.5*

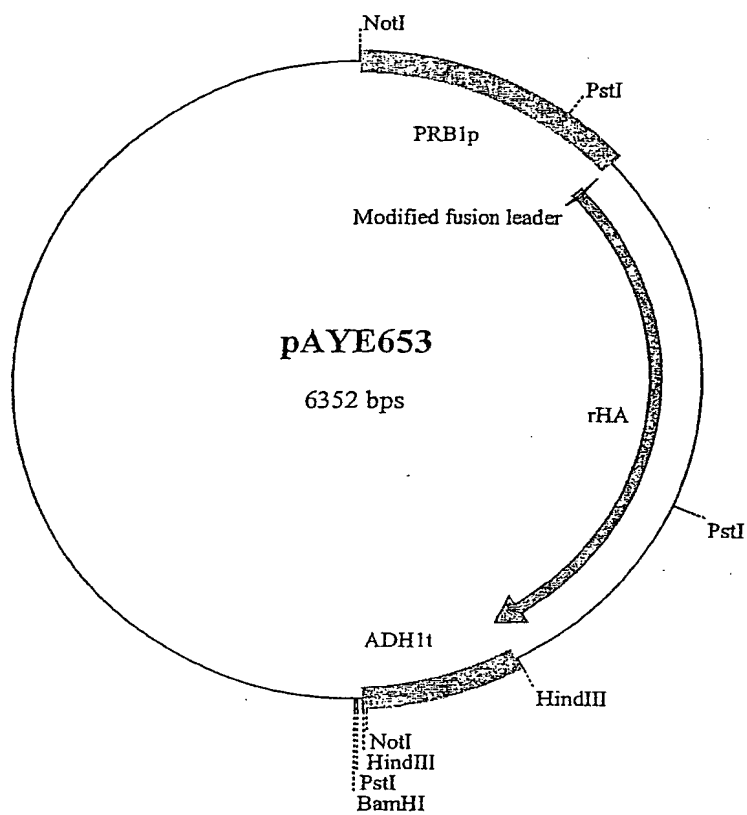
*Fig.6*

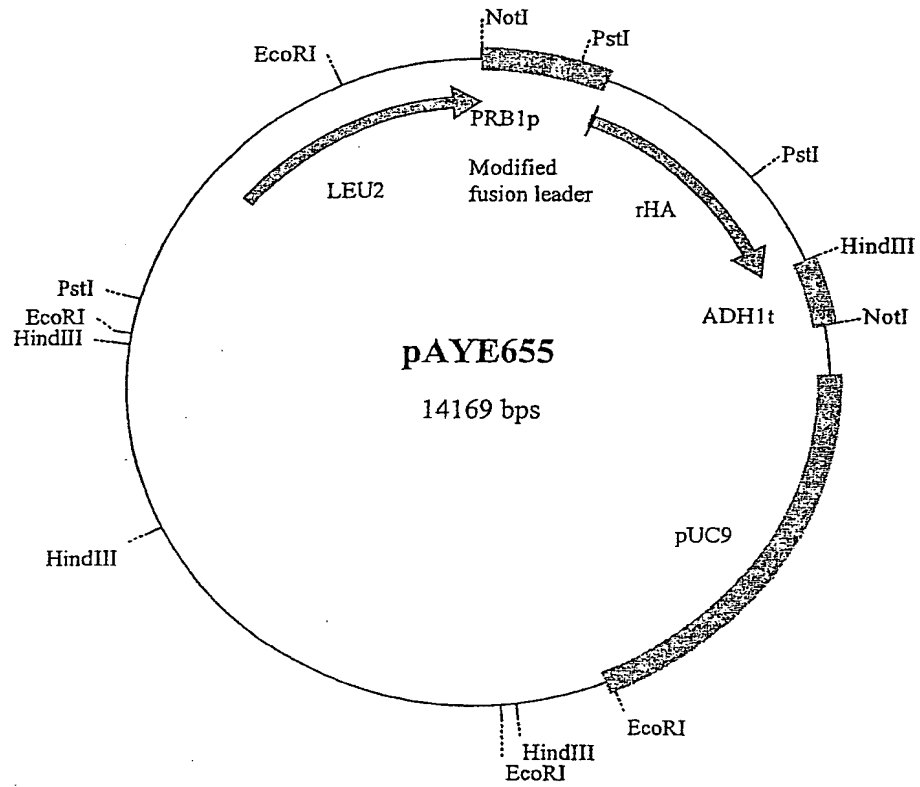
**Fig. 7**

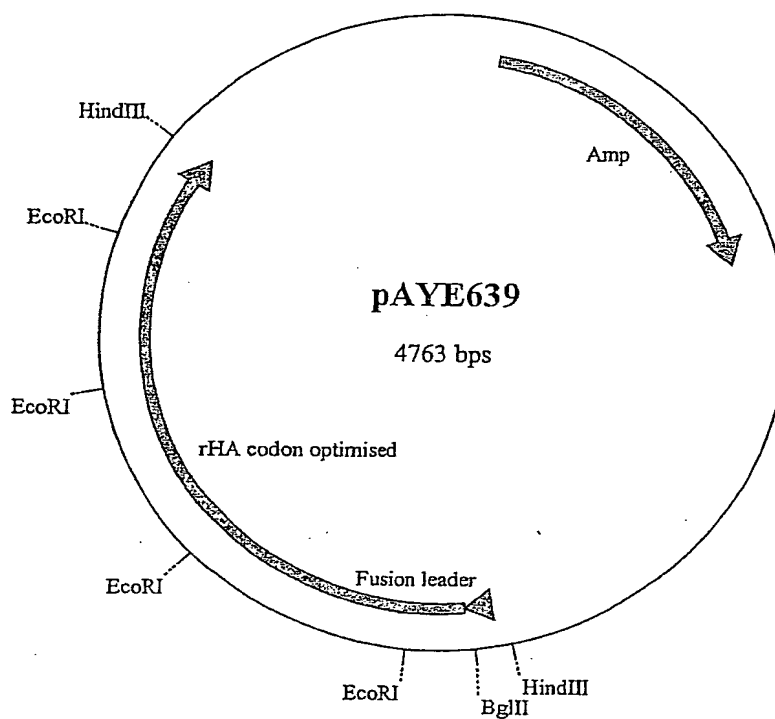
*Fig. 8*



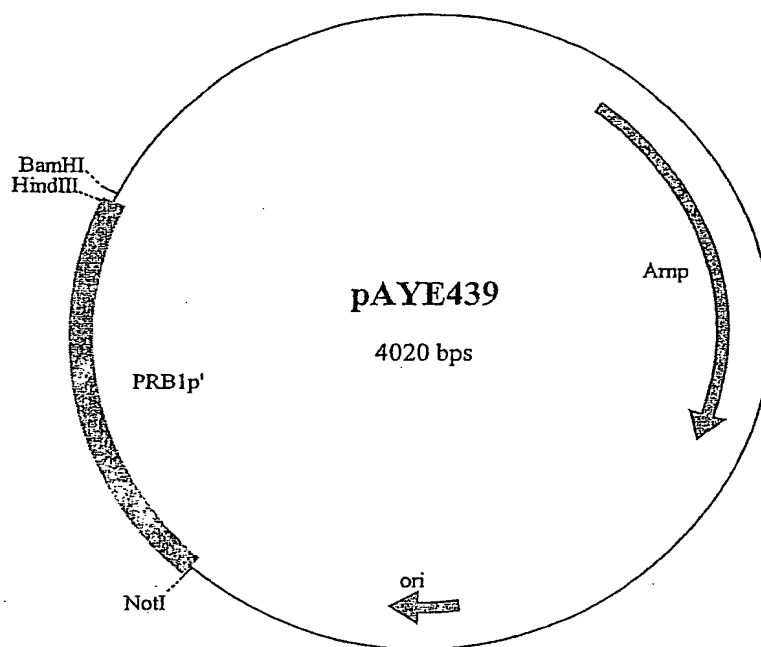
**Fig. 9**

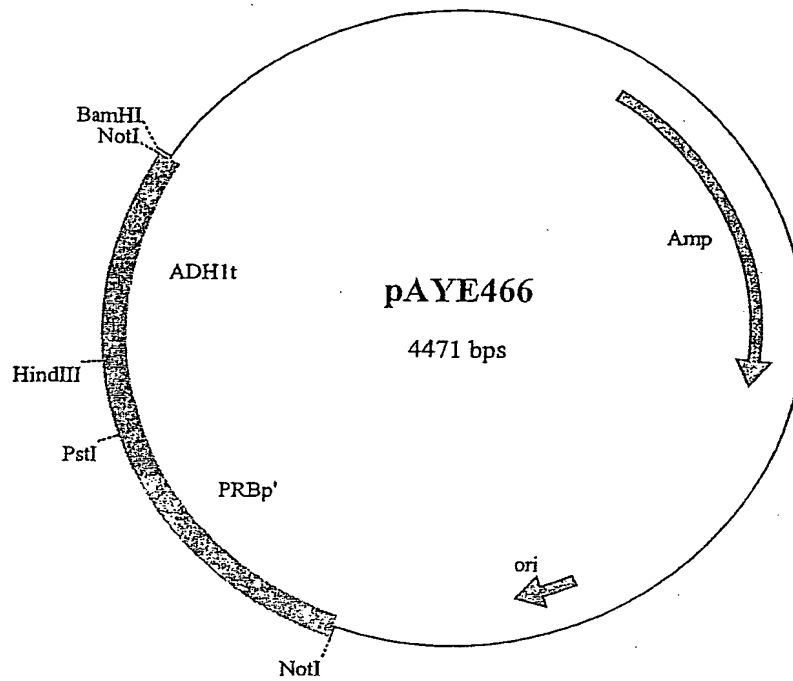


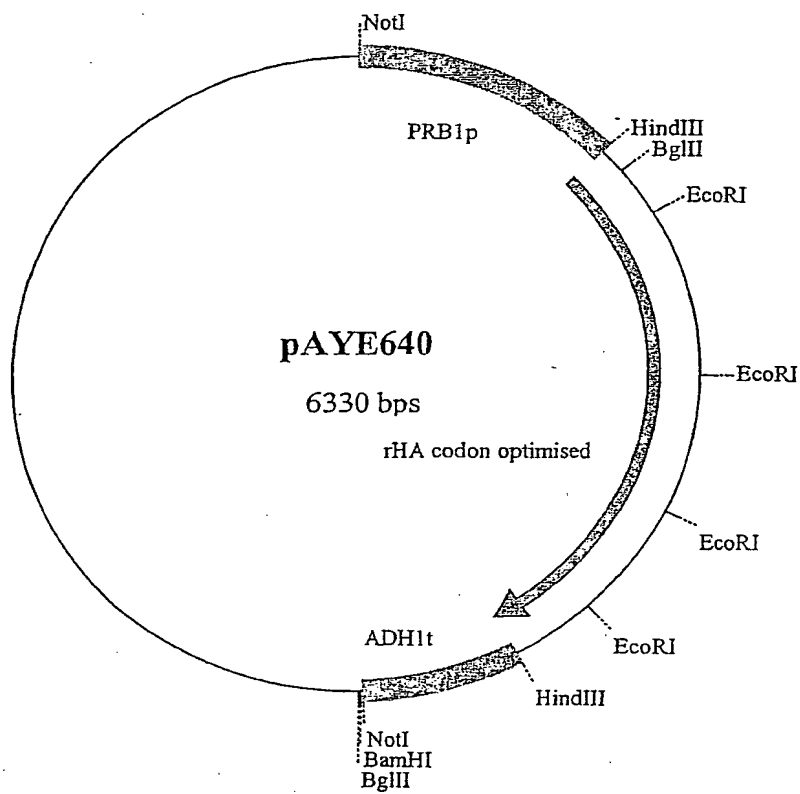
*Fig.10*

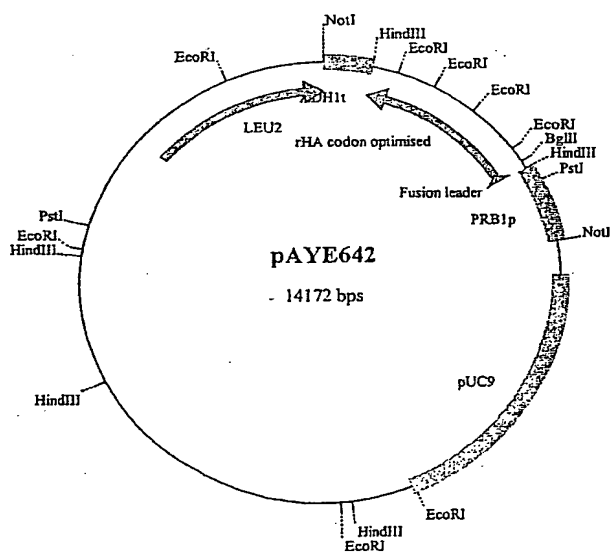
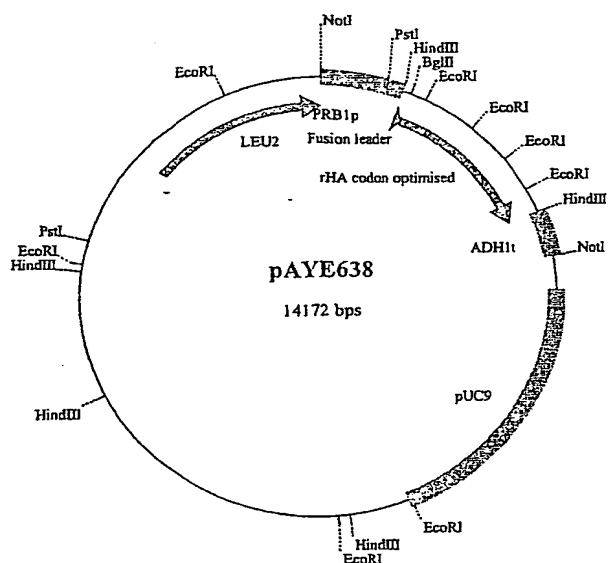
*Fig.11*

*Fig.12*

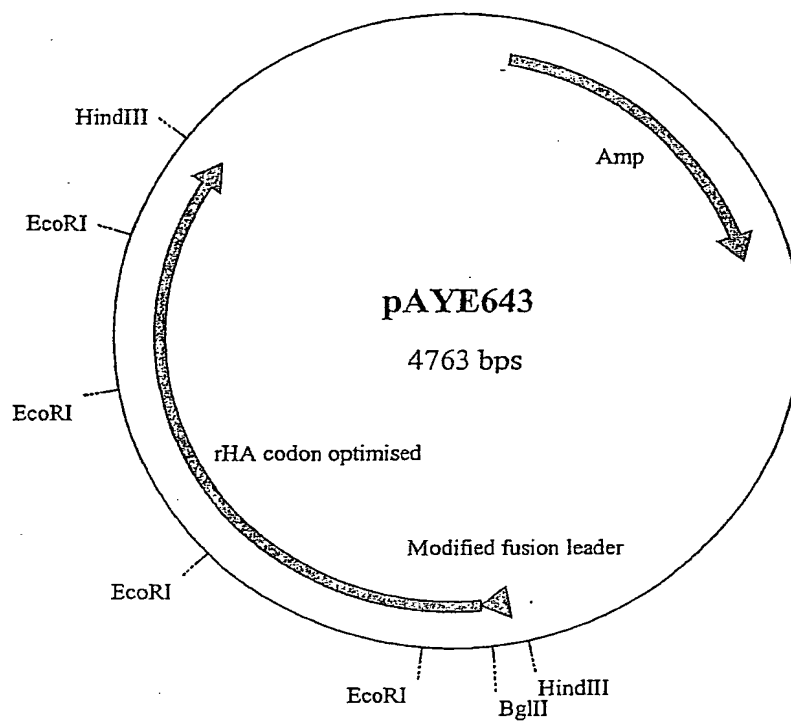


*Fig.13*

*Fig.14*

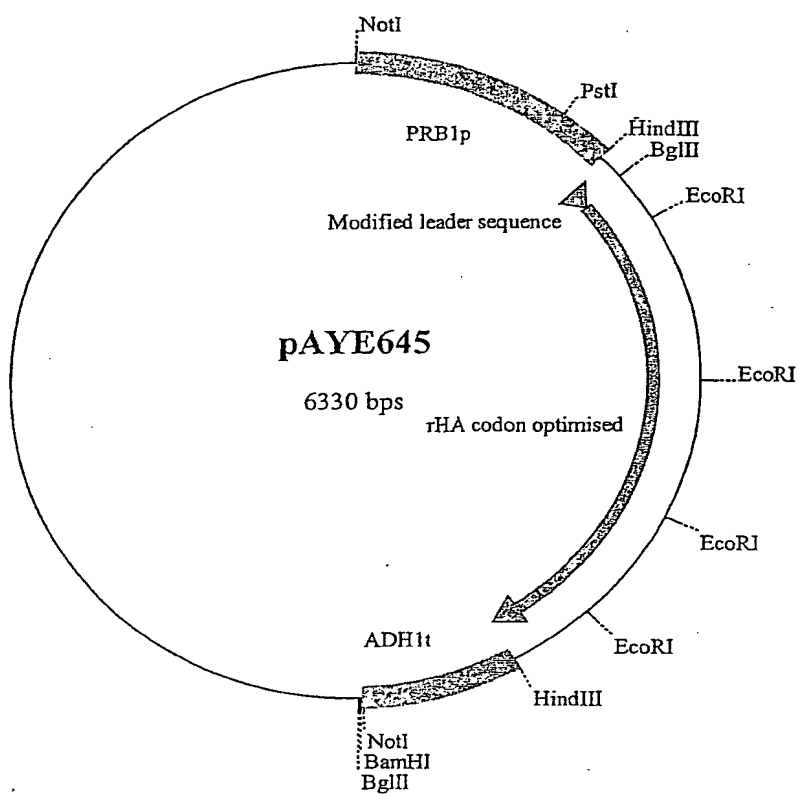
*Fig.15*

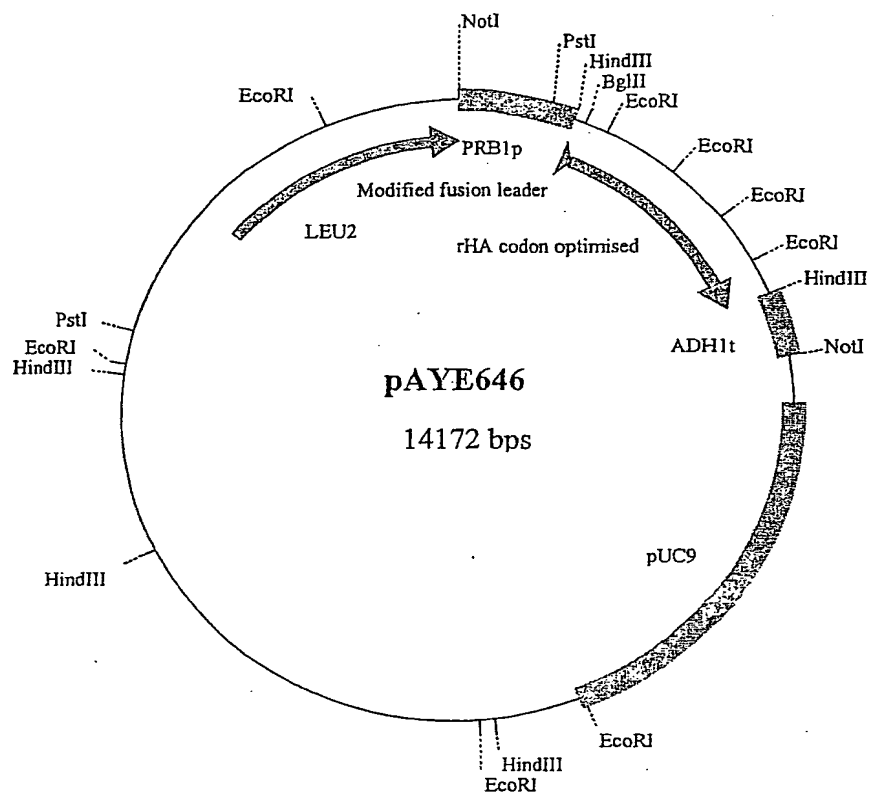
**Fig.16**





**Fig.17**



*Fig.18*

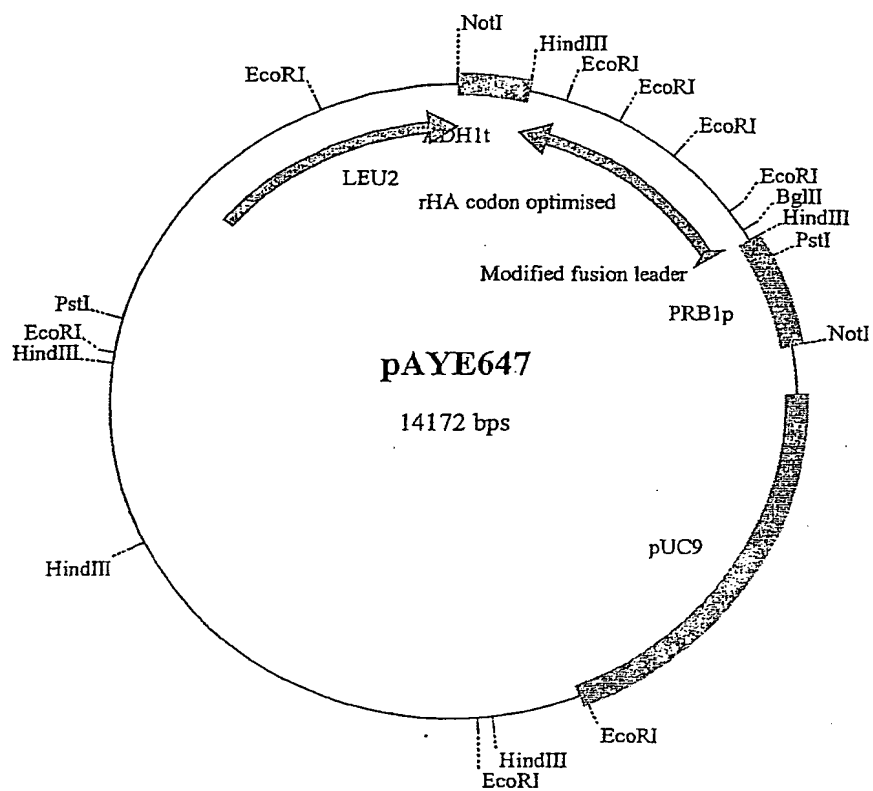
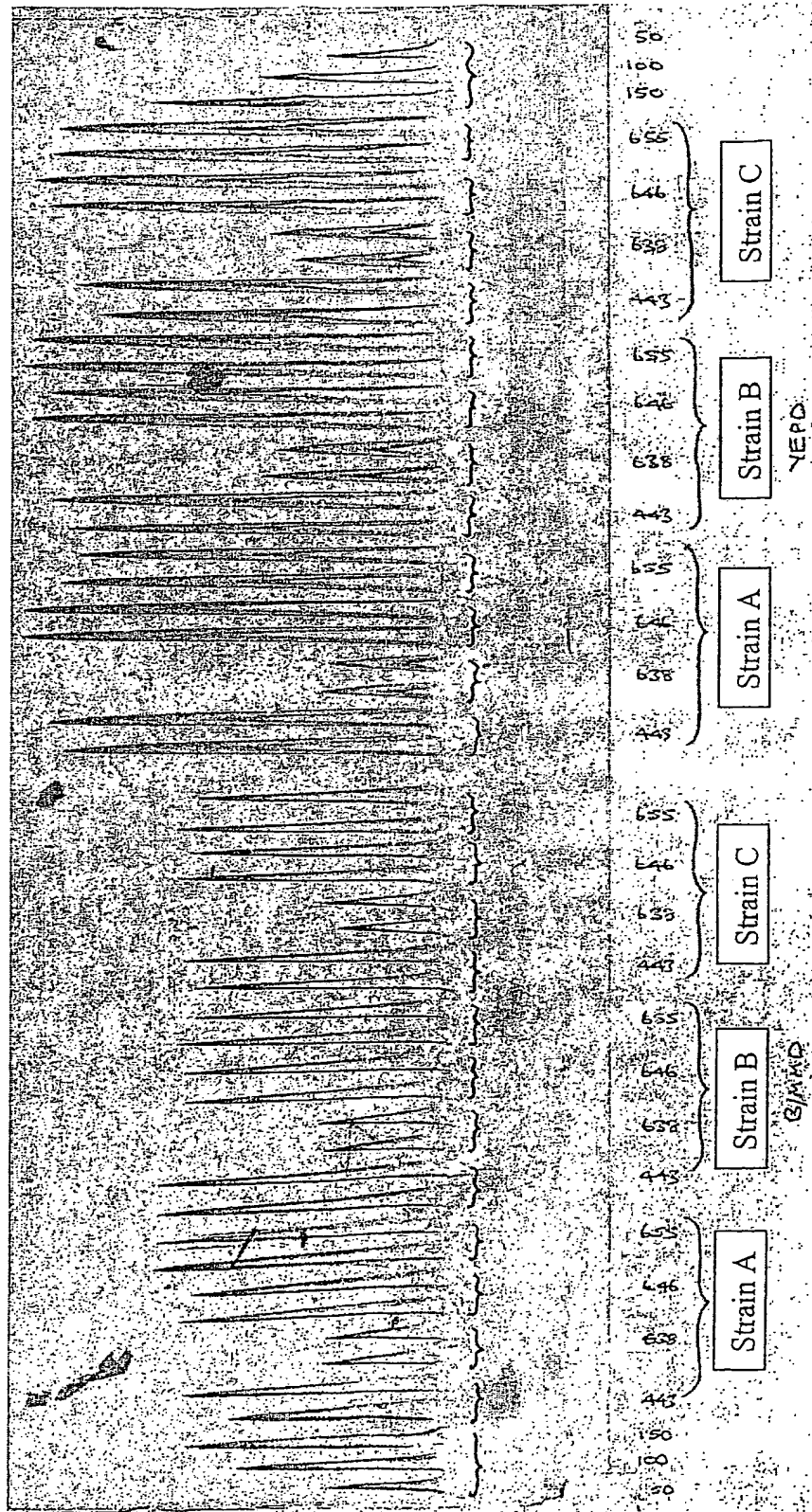
*Fig.19*

Fig. 20



*Fig.21*

Strain	Plasmid	Yx/s (g/g)	Yp/s (mg/g)	rHA (g/L)
C	pAYE443 (1 <sup>st</sup> feeds)	0.33	10.4	2.9
	(fill and draw)	0.34	11.5	3.2
	pAYE638 (1 <sup>st</sup> feeds)	0.36	*	*
	(fill and draw)	0.36	2.4	0.7
	pAYE646 (1 <sup>st</sup> feeds)	0.33	11.6	3.2
	(fill and draw)	0.35	12.2	3.5
	pAYE655 (1 <sup>st</sup> feeds)	0.37	12.1	3.4
	(fill and draw)	0.35	13.0	3.7
B	pAYE443 (1 <sup>st</sup> feeds)	0.35	10.5	2.8
	pAYE646 (1 <sup>st</sup> feeds)	0.35	13.0	3.5
	(fill and draw)	0.33	12.8	3.6

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Fig.22

Plasmid designation	Leader	Desired Protein
pAYE443 pAYE467	HSA/MF $\alpha$ -1 natural codon bias	HSA natural codon bias
pAYE655 pAYE643	modified HSA/MF $\alpha$ -1 FIVSI fully codon biased the rest has natural codon bias	HSA natural codon bias
pAYE638 pAYE639 pAYE640 pAYE642	HSA/MF $\alpha$ -1 all fully codon biased	HSA fully codon biased
pAYE645 pAYE646 pAYE647	modified HSA/MF $\alpha$ -1 all fully codon biased	HSA fully codon biased

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